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Research Article

Hidden or inadvertent illegal amphibian trade? Genetic analyses reveal *Quasipaa spinosa* misidentified as *Hoplobatrachus chinensis* in the trade

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Abstract

Amphibian misidentification within the commercial wildlife trade represents a growing challenge for biodiversity conservation and wildlife regulation. Morphologically similar species are frequently traded under incorrect names, which can obscure conservation status, facilitate illegal trade, and mislead consumers. We purchased four specimens advertised as *Hoplobatrachus chinensis,* a widespread farmed species, from an online marketplace in China. Because of their unexpected morphology upon reception, we barcoded them with a widely used tyrosinase (*TYR*) gene marker. We reconstructed a Bayesian inference-based phylogeny using a final dataset consisting of 572 bp from 51 homologous *TYR* sequences. The resulting tree clustered all four samples within the *Quasipaa spinosa* clade with high posterior probabilities (>0.99), rather than *H. chinensis* as advertised. This finding indicates that a protected species has been misidentified and traded despite China's wildlife trade regulations. The case underscores the urgent need for accurate taxonomic identification tools for traders to prevent such misidentifications and stricter amphibian trade regulations to curb the ongoing illegal trade of threatened taxa.

Key words: Amphibian trade, Chinese spiny frog, species identity discrepancy, Tyrosinase barcoding, wildlife conservation

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Introduction

Amphibians are one of the most threatened vertebrate groups globally, with over 41% of amphibian species currently classified as threatened with extinction (Luedtke et al. 2023). These threats are driven by a combination of habitat destruction, climate change, emerging infectious diseases, in synergy with unregulated exploitation (Kolby et al. 2024; Borzée et al. 2025), including that of widespread species (Othman et al. 2024). Across Asia, exploitation for consumption, traditional medicine, and the wildlife trade continue to be a primary driver of amphibian population declines (Kitade et al. 2022; Dai et al. 2025).

In China, amphibians have been traded both domestically and internationally for centuries (Meng et al. 2016; Li et al. 2021; Lu et al. 2025). In recent decades, however, the rapid expansion of online commerce platforms has profoundly reshaped the dynamics of the wildlife trade (Sung et al. 2018). Digital marketplaces such as Taobao, WeChat, and other e-commerce platforms have facilitated the easy purchase and transport of live amphibians (Ji et al. 2020), often in the absence of appropriate permits or scientific oversight (Wang et al. 2025). Although such platforms officially prohibit the sale of protected wildlife (Yao 2023), amphibians are frequently listed under vague or incorrect trade names, complicating effective enforcement. Species misidentification in the wildlife trade can lead to multiple adverse consequences for biodiversity conservation and regulatory enforcement. First, species misidentification allows the illegal or mistaken sale of protected taxa under erroneous labels, thereby undermining conservation legislation and enforcement (Sung et al. 2018). Second, species misidentification can result in inaccurate records of species occurrence and trade volumes, which in turn compromise management decisions and population assessments (Hinsley et al. 2023). Consequently, developing accurate and cost-effective molecular identification methods for traded amphibians is essential for both conservation and biosecurity.

The genus *Quasipaa* Dubois, 1992 (family Dicroglossidae) includes relatively large, stream-associated frogs occurring across southern and southwestern China, and extending southward into central Vietnam, Laos, Thailand, and Cambodia (Frost 2025). However, overexploitation and habitat degradation have resulted in significant population declines (Song et al. 2025), particularly for species that are important in the trade for human food. Among them, Chinese spiny frog (*Quasipaa spinosa*) is one of the largest species and is widely consumed as a delicacy and collected for the wildlife trade (Hu et al. 2017). The species is currently listed as Vulnerable on both the International Union for Conservation of Nature (IUCN) Red List (2020 assessment; ASG 2023) and the Red List of Chinese Species (Chan et al. 2014). Members of this genus are ecologically and morphologically similar, with overlapping coloration patterns and morphology that can easily lead to confusion in identification (Fei et al. 2012), not only between conspecifics but also with other members of the Dicroglossidae family. Therefore, the misidentification of *Quasipaa* species, particularly in the trade, is not surprising given their morphological similarity to other dicroglossid frogs such as East Asian Bullfrog (*Hoplobatrachus chinensis*). The latter is another common species widely farmed for food and trade (Lee et al. 2022), making it a convenient label under which other taxa may be sold. Yet, the substitution of threatened species for more common ones represents a serious conservation issue.

In this study, we report a case of four frogs sold online as "*Hoplobatrachus chinensis*", which were molecularly identified as *Quasipaa spinosa*. To confirm species identity, we combine morphological observation with molecular identification and phylogenetic barcoding of the *TYR* gene fragments. This fragment is widely used for Asian dicroglossid identification (Kotaki et al. 2010; Yu et al. 2015). We also discuss the broader implications of such misidentifications for amphibian conservation, law enforcement, and consumer awareness in China's expanding online wildlife trade.

Materials and methods

DNA extraction, amplification, and sequencing

Four "*Hoplobatrachus chinensis*" individuals were purchased from an online retailer on Taobao (https://www.taobao.com). The frogs were shipped labelled as "ornamental amphibians" without any scientific name. Upon arrival, the specimens were photographed, and basic morphological traits such as body colour, skin texture, and limb proportions were documented (AmphibiaChina 2025; Fig. 2). The specimen acquisition and research were conducted under the Institutional Animal Care and Use Committee (**IACUC**) number 2025028 provided by the ethic board of Nanjing Forestry University.

Genomic DNA was extracted from the thigh muscle tissues of the four specimens using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germany) following the protocol of the manufacturer. For all samples, we sequenced a 530-bp fragment of the tyrosinase exon 1 (*TYR*) nuclear gene using the primer pair Tyr 1A (5'-AGGTCCTCTTRAGCAAGGAATG-3') Tyr 1E (5'- GAGAAGAAAGAW-GCTGGGCTGAG-3'; (Bossuyt et al. 2000). We amplified all fragments in a total reaction volume of 20 µl, consisting of 10 µl of 2× Hieff HotStart PCR Genotyping Master Mix (with dye), 1.0 µl of each primer, 1.0 µl of DNA template, and 7 µl of ddH_2O. Thermal profiles for the polymerase chain reactions (PCR) were as follows: initial denaturation at 95 °C for 4 minutes, followed by 35 cycles of denaturation at 94 °C for 20s, annealing at 55 °C for *TYR* for 20s, and extension at 72 °C for 1.5 min. The cycles were followed by a 10-minute final extension at 72 °C. PCR amplifications, amplicon purification and double reads sequencing for all samples were performed by Sangon Biotech Co., Ltd. (Shanghai, China).

Sequence analysis and phylogeny reconstruction

Raw sequences were trimmed in Geneious Prime 2025.0.2 (https://www.geneious.com). Using Clustal W algorithm implemented in Geneious, we aligned all four sequences with their most homologous sequences retrieved from NCBI (www.ncbi.nlm.nih.gov). Alignments were manually checked to remove ambiguous regions. The final sequence alignment consisted of 572 bp of 51 *TYR* sequences representing *Quasipaa spinosa* individuals and its congeners.

Phylogenetic relationships were inferred using Bayesian inference (BI) implemented in MrBayes 3.2.7 (Ronquist et al. 2012). The dataset was partitioned by three codon position strategy into two subsets, with the best-fit substitution models selected by PartitionFinder, GTR+G for codon1 and codon3 combined, and GTR for codon2. The Markov Chain Monte Carlo (MCMC) analysis was performed for 10,000,000 generations (ngen = 10000000) with four chains (nchains = 4) and a chain temperature of 0.2 (temp = 0.2). Trees were sampled for every 1,000 generations. We used a random starting tree and a fixed seed of 95478, saving the branch lengths. Posterior probabilities were calculated after discarding the burn-in portion of the trees. We assessed the convergence of the analyses by examining the average standard deviation of split frequencies between independent runs to be lower than 0.05, and we ensured the effective sample size (ESS) for all parameters exceeded 200

(Ronquist et al. 2019). These diagnostics were conducted using Tracer v.1.7.2 software (Rambaut et al. 2018), which confirmed that MCMC chains had adequately converged and that parameter estimates were based on sufficiently sampled posterior distributions.

Results

All four specimens yielded high-quality *TYR* sequences approximately 572 bp in length. Sequence comparisons revealed that the samples shared over 99% identity with published *Quasipaa spinosa* sequences (Fig. 1). The Bayesian Inference phylogeny based on the *TYR* sequences alignment (Fig. 1) recovered seven well-supported clades corresponding to distinct *Dicroglossidae* species: *Fejervarya* sp., *Hoplobatrachus* sp., *Q. jiulongensis*, *Q. exilispinosa*, *Q. spinosa*, *Q. shini*, and *Q. yei*, (Fig. 1). The tree clustered all four purchased specimens (labelled as 25QsC001–25QsC004) unambiguously within the clade containing homologous sequences of *Q. spinosa* with high posterior probabilities (>0 .99). This confirmed their molecular identification as *Quasipaa spinosa* and not *H. chinensis*.

Although the frogs exhibited a general body shape similar to *H. chinensis*, several diagnostic morphological features were consistent with *Q. spinosa*, including relatively rough dorsal and lateral skin, tubercles on the chest with small round warts scattered between them, each typically bearing a small black spine, and a pale-yellow ventral surface, with the throat and limbs either

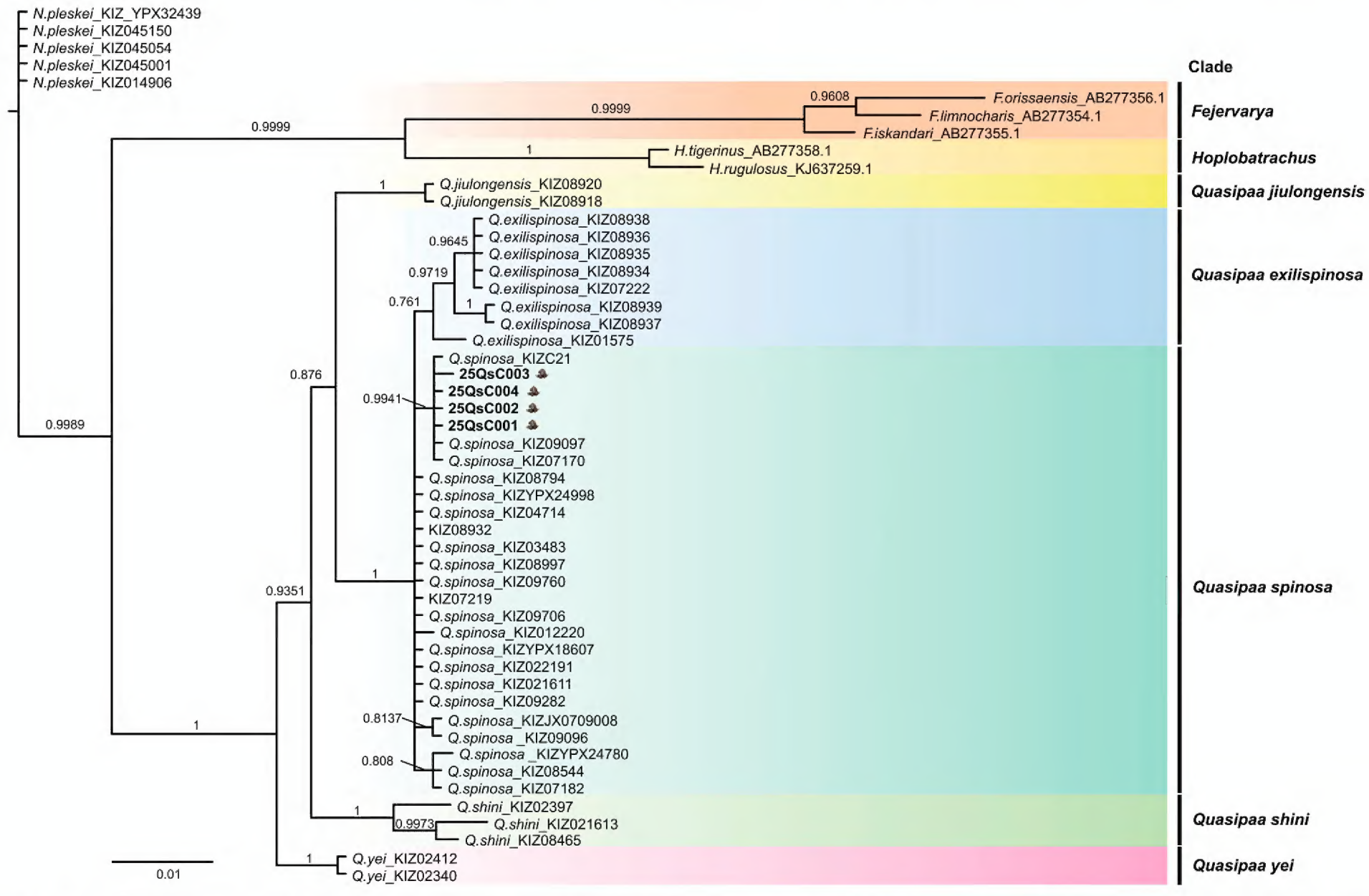

Figure 1. Bayesian phylogenetic tree based on *TYR* gene fragment sequences showing relationships among *Quasipaa* species. Node values indicate posterior probabilities. The frog miniatures next to the samples represent the individuals that were purchased.

Figure 2. Four *Quasipaa spinosa* purchased online under the false visual of *Hoplobatrachus chinensis*, a farmed species. Panels correspond to individual specimens 25QsC001–25QsC004, each showing dorsal (left) and ventral (right) views with a ruler for scale. Snout–vent length (SVL) measurements are as follows: 25QsC001 (95 mm), 25QsC002 (104 mm), 25QsC003 (101 mm), and 25QsC004 (97 mm). All specimens exhibit dark dorsal coloration and brown cloud-like spots on the ventral surfaces of the limbs. In addition, conspicuous chest tubercles are visible in 25QsC001, 25QsC003, and 25QsC004, with small round warts between the tubercles bearing distinct black spines.

unmarked or exhibiting brown cloud-like spots (AmphibiaChina 2025; Fig. 2). Based on these morphological traits combined with molecular evidence, we could confirm that the four purchased specimens were *Q. spinosa*.

Discussion

Misidentification in amphibian commerce

The misidentification of amphibians in the trade has long posed a major challenge to effective wildlife monitoring and regulation (Chen 2023). Many species exhibit convergent morphological traits such as cryptic coloration, similar size ranges, or overlapping habitats, which makes visual identification unreliable (Liu et al. 2018). In particular, members of the genera *Quasipaa* and *Hoplobatrachus* are large-bodied frogs with mottled skin and similar dorsal colour patterns, making them easily confused by non-specialists (Ding et al. 2022).

This cryptic morphology has profound implications for biodiversity monitoring and regulatory enforcement (Fernandez et al. 2025). When species are

mislabelled or misidentified, the resulting data on trade volumes and species distributions becomes unreliable, compromising both national and international conservation assessments. Threatened or protected taxa may be traded as common species, leading to unrecorded extraction from the wild and unnoticed population declines (Ji et al. 2020; Hinsley et al. 2023). Consequently, conservation priorities derived from flawed datasets risk being misdirected, thereby weakening the effectiveness of policy interventions and the allocation of limited conservation resources (Hughes et al. 2021).

Within the wildlife trade system, misidentification is not only an accidental error but also reflects deeper socio-economic and regulatory dynamics. Collectors and intermediaries often lack taxonomic expertise, while economic incentives encourage traders to obscure species identities to evade detection or enhance market appeal (Liew et al. 2021; Othman et al. 2022). The expansion of e-commerce platforms has further exacerbated the problem, as ambiguous listings, inconsistent use of scientific names, and poor traceability facilitate the circulation of protected species under misleading or generic labels (Ji et al. 2020). Such practices obscure the true extent of wildlife exploitation, impede enforcement efforts, and create loopholes that allow wild-caught individuals to be laundered as captive-bred stock (Sung et al. 2024). These dynamics intensify harvesting pressure on vulnerable species and accelerate the decline of amphibian biodiversity (Dai et al. 2025).

Quasipaa spinosa is an ecologically and economically significant amphibian that plays a vital role in maintaining stream ecosystem balance while remaining highly sensitive to environmental disturbance (Hou et al. 2023; Song et al. 2025). Over-collection for consumption and trade has led to a dramatic decline in the wild population of *Q. spinosa* (ASG 2023). The presence of *Q. spinosa* in the wildlife trade, especially under misleading names, indicates that wild populations may be subjected to unmonitored harvesting. Continued collection could exacerbate population decline, especially in isolated montane habitats. Trade regulation and enforcement are therefore required for the species, taking into account the possible impact on the livelihood of the population traditionally benefitting from the harvest of the species (Borzée et al. 2021).

Integrated role of molecular tools and public engagement in amphibian conservation

In this study, *Quasipaa* exhibits cryptic morphology, and relying solely on physical traits can easily lead to misidentification, such as confusing it with *Hoplobatrachus*. Therefore, integrating molecular diagnostics into customs inspections and online marketplace monitoring could greatly enhance enforcement efficiency and traceability (Hughes et al. 2021). To support this development, government agencies should collaborate with academic institutions to establish comprehensive genetic reference libraries for commonly traded amphibians (Rehman et al. 2015; Ratnasingham et al. 2024), while e-commerce platforms could be required to provide a clear labelling of the identity of animal listings.

Beyond technological solutions, effective conservation also depends on communication among scientists, policymakers, and the public. Many amphibian enthusiasts remain unaware of species-level distinctions or relevant legal

frameworks (e.g., CITES regulations; https://www.cites.org), which can lead to inadvertent illegal purchases. Public outreach initiatives, including open-access databases with accurate identification guides, photographs, and regulatory information, could improve awareness and compliance (WWF 2024). More broadly, molecular monitoring contributes to a growing understanding of hidden aspects of the wildlife trade that traditional enforcement is often not equipped to identify. Documenting such cases provides essential evidence to inform policy reform, strengthen trade regulation, and prioritize conservation action.

Conclusions

Our study documents the online sale of the Vulnerable species *Quasipaa spinosa* in China under the misleading label "*Hoplobatrachus chinensis*", with *TYR* gene–based molecular analysis confirming the true identity of the specimens and revealing a clear case of misidentification. These findings underscore the urgent need for genetic verification in wildlife trade monitoring and highlight the hidden threats posed by online markets to amphibian conservation. Integrating molecular tools with trade policy enforcement, consumer education, and habitat protection will be critical to prevent further declines of threatened species such as *Q. spinosa* due to unregulated commerce.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

The specimen acquisition and research were conducted under the Institutional Animal Care and Use Committee (IACUC) number 2025028 provided by the ethic board of Nanjing Forestry University.

Use of AI

No use of AI was reported.

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Author contributions

Xiaoli Zhang: Writing – original draft, Visualisation, Writing – review and editing, Validation, Supervision, Methodology, Software, Formal analysis, Conceptualisation. Amaël Borzée: Writing – review and editing, Resources, Conceptualisation, Visualisation, Supervision, Project administration, Funding Acquisition. Sumbul Gill: Writing – review and editing, Methodology, Supervision, Software. Siti N. Othman: Writing – review and editing, Methodology, Software, Formal analysis, Conceptualisation, Resources, Visualisation, Supervision, Project administration, Funding Acquisition. All authors contributed equally to the final version of the manuscript.

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Data availability

All DNA sequences generated in this study have been submitted to the GenBank (https://www.ncbi.nlm.nih.gov/), under the following accession numbers: 25QsC001 (PX781737), 25QsC002 (PX781738), 25QsC003 (PX781739), and 25QsC004 (PX781740).

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